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This is a request for filing a PROVISIONAL APPLICATION under 37 CFR §1.53(c)(1).

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TITLE
COMPOSITIONS AND METHODS FOR TOPICAL APPLICATION AND TRANSDERMAL DELIVERY OF BOTULINUM TOXINS

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Respectfully submitted,

Signature Joseph D. Eng Jr. Date March 3, 2004

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PROVISIONAL PATENT APPLICATION

COMPOSITIONS AND METHODS FOR TOPICAL APPLICATION AND TRANSDERMAL DELIVERY OF BOTULINUM TOXINS

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BACKGROUND OF THE INVENTION

[0001] This invention relates to new compositions comprising a botulinum toxin, more specifically to such compositions that enable the transport or delivery of a botulinum toxin through the skin or epithelium (also referred to as "transdermal delivery"), and that therefore may be used as topical applications for providing a botulinum toxin to a subject, for various therapeutic, aesthetic and/or cosmetic purposes, as described herein.

[0002] Botulinum toxins (also known as botulin toxins or botulinum neurotoxins) are neurotoxins produced by the gram-positive bacteria *Clostridium botulinum.* They act to produce paralysis of muscles by preventing synoptic transmission or release of acetylcholine across the neuromuscular junction, and are thought to act in other ways as well. Their action essentially blocks signals that normally would cause muscle spasms or contractions, resulting in paralysis.

[0003] Botulinum toxin is classified into eight neurotoxins that are serologically related, but distinct. Of these, seven can cause paralysis, namely botulinum neurotoxin serotypes A, B, C, D, E, F and G. Each of these is distinguished by neutralization with type-specific antibodies. Nonetheless, the molecular weight of the botulinum toxin protein molecule, for all seven of these active botulinum toxin serotypes, is about 150 kD. As released by the bacterium, the botulinum toxins are complexes comprising the 150 kD botulinum toxin protein molecule in question along with associated non-toxin proteins. The botulinum toxin type A complex can be produced by Clostridia bacterium as 900 kD, 500 kD and 300 kD forms. Botulinum toxin types B and C are apparently produced as only a 700 kD or 500 kD complex. Botulinum toxin type D is produced as both 300 kD and 500 kD complexes. Botulinum toxin types E and F are produced as only approximately 300 kD complexes. The complexes (i.e. molecular weight greater than about 150 kD) are believed to contain a non-toxin hemaglutinin protein and a non-toxin and non-toxic nonhemaglutinin protein. These two non-toxin proteins (which along with the botulinum toxin molecule comprise the relevant neurotoxin complex) may act to provide stability against denaturation to the botulinum toxin molecule and protection against digestive acids when toxin is ingested. Additionally, it is possible that the larger (greater than about 150 kD molecular weight) botulinum toxin complexes may result in a slower rate of diffusion of the botulinum toxin away from a site of intramuscular injection of a botulinum toxin complex.

[0004] The different serotypes of botulinum toxin vary in the animal species that they affect and in the severity and duration of the paralysis they evoke. For example, it has been determined that botulinum toxin type A is 500 times more potent, as measured by the rate of paralysis produced in the rat, than is botulinum toxin type B. Additionally, botulinum toxin type B has been determined to be non-toxic in primates at a dose of 480 U/kg, about 12 times the primate LD_{50} for type A. Due to the molecule size and molecular structure of botulinum toxin, it cannot cross stratum corneum and the multiple layers of the underlying skin architecture.

[0005] Botulism, the characteristic symptom complex from systemic botulinum toxin exposure, has existed in Europe since antiquity. In 1895, Emile P. van Ermengem first isolated the anaerobic spore-forming bacillus from raw salted pork meat obtained from post-mortem tissue of victims who died of botulism in Belgium. Van Ermengem found the disease to be caused by an extracellular toxin that was produced by what he called Bacillus botulinus (Van Ermengem, Z Hyyg Infektionskr, 26:1-56; Rev Infect (1897)). The name was changed in 1922 to Clostridium botulinum. The name Clostridium was used to reflect the anaerobic nature of the microorganism and also its morphologic characteristics (Carruthers and Carruthers, Can J Ophthalmol, 31:389-400 (1996)). In the 1920's, a crude form of Botulinum toxin type A was isolated after additional outbreaks of food poisoning. Dr. Herman Sommer at the University of California, San Francisco made the first attempts to purify the neurotoxin (Borodic et al., Ophthalmic Plast Recostr Surg, 7:54-60 (1991)). In 1946, Dr. Edward J. Schantz and his colleagues isolated the neurotoxin in crystalline form (Schantz et al., In: Jankovi J, Hallet M (Eds) Therapy with Botulinum Toxin, New York, NY: Marcel Dekker, 41-49 (1994)). By 1949, Burgen and his associates were able to demonstrate that the Botulinum toxin blocks impulses across the neuromuscular junction (Burgen et al., J Physiol, 109:10-24 (1949)). Allan B. Scott first used botulinum toxin A (BTX-A) in monkeys in 1973. Scott demonstrated reversible ocular muscle paralysis lasting 3 months (Lamanna, Science, 130:763-772 (1959)). Soon afterwards, BTX-A was reported to be a successful treatment in humans for strabismus, blepharospasm, and spasmodic torticollis (Baron et al., In: Baron EJ, Peterson LR, Finegold SM (Eds), Bailey & Scotts Diagnostic Microbiology, St. Louis, MO: Mosby Year Book, 504-523 (1994); Carruthers and Carruthers, Adv Dermatol, 12:325-348 (1997); Markowitz, In: Strickland GT (Eds) Hunters Tropical Medicine, 7th ed. Philadelphia: W.B. Saunders, 441-444 (1991)). In 1986, Jean and Alastair Carruthers, a husband and wife team consisting of an ocuplastic surgeon and a dermatologist,

began to evolve the cosmetic use of BTX-A for treatment of movement-associated wrinkles in the glabella area (Schantz and Scott, In Lewis GE (Ed) Biomedical Aspects of Botulinum, New York: Academic Press, 143-150 (1981)). The Carruthers' use of BTX-A for the treatment of wrinkles led to their seminal publication of this approach in 1992 (Schantz and Scott, In Lewis GE (Ed) Biomedical Aspects of Botulinum, New York: Academic Press, 143-150 (1981)). By 1994, the same team reported experiences with other movement-associated wrinkles on the face (Scott, Ophthalmol, 87:1044-1049 (1980)). This in turn led to the birth of the era of cosmetic BTX-A treatment.

[0006] Skin protects the body's organs from external environmental threats and acts as a thermostat to maintain body temperature. It consists of several different layers, each with specialized functions. The major layers include the epidermis, the dermis and the hypodermis. The epidermis is a stratifying layer of epithelial cells that overlies the dermis, which consists of connective tissue. Both the epidermis and the dermis are further supported by the hypodermis, an internal layer of adipose tissue.

[0007] The epidermis, the topmost layer of skin, is only 0.1 to 1.5 millimeters thick (Inlander, Skin, New York, NY: People's Medical Society, 1-7 (1998)). It consists of keratinocytes and is divided into several layers based on their state of differentiation. The epidermis can be further classified into the stratum corneum and the viable epidermis, which consists of the granular melphigian and basal cells. The stratum corneum is hygroscopic and requires at least 10% moisture by weight to maintain its flexibility and softness. The hygroscopicity is attributable in part to the water-holding capacity of keratin. When the horny layer loses its softness and flexibility it becomes rough and brittle, resulting in dry skin.

[0008] The dermis, which lies just beneath the epidermis, is 1.5 to 4 millimeters thick. It is the thickest of the three layers of the skin. In addition, the dermis is also home to most of the skin's structures, including sweat and oil glands (which secrete substances through openings in the skin called pores, or comedos), hair follicles, nerve endings, and blood and lymph vessels (Inlander, Skin, New York, NY: People's Medical Society, 1-7 (1998)). However, the main components of the dermis are collagen and elastin.

[0009] The hypodermis is the deepest layer of the skin. It acts both as an insulator for body heat conservation and as a shock absorber for organ protection (Inlander, Skin, New York, NY: People's Medical Society, 1-7 (1998)). In addition, the hypodermis also stores fat for

energy reserves. The pH of skin is normally between 5 and 6. This acidity is due to the presence of amphoteric amino acids, lactic acid, and fatty acids from the secretions of the sebaceous glands. The term "acid mantle" refers to the presence of the water-soluble substances on most regions of the skin. The buffering capacity of the skin is due in part to these secretions stored in the skin's horny layer.

[0010] Wrinkles, one of the telltale signs of aging, can be caused by biochemical, histological, and physiologic changes that accumulate from environmental damage (Benedetto, International Journal of Dermatology, 38:641-655 (1999)). In addition, there are other secondary factors that can cause characteristic folds, furrows, and creases of facial wrinkles (Stegman et al., The Skin of the Aging Face Cosmetic Dermatological Surgery, 2nd ed., St. Louis, MO: Mosby Year Book: 5-15 (1990)). These secondary factors include the constant pull of gravity, frequent and constant positional pressure on the skin (i.e., during sleep), and repeated facial movements caused by the contraction of facial muscles (Stegman et al., The Skin of the Aging Face Cosmetic Dermatological Surgery, 2nd ed., St. Louis, MO: Mosby Year Book: 5-15 (1990)). Different techniques have been utilized in order potentially to mollify some of the signs of aging. These techniques range from facial moisturizers containing alpha hydroxy acids and retinol to surgical procedures and injections of neurotoxins.

[0011] One of the principal functions of skin is to provide a barrier to the transportation of water and substances potentially harmful to normal homeostasis. The body would rapidly dehydrate without a tough, semi-permeable skin. The skin helps to prevent the entry of harmful substances into the body. Although most substances cannot penetrate the barrier, a number of strategies have been developed to selectively increase the permeability of skin with variable success.

[0012] Botulinum toxin type A is said to be the most lethal natural biological agent known to man. Spores of *C. botulinum* are found in soil and can grow in improperly sterilized and sealed food containers. Ingestion of the bacteria can cause botulism, which can be fatal. At the same time, the muscle-paralyzing effects of botulinum toxin have been used for therapeutic effects. Controlled administration of botulinum toxin has been used to provide muscle paralysis to treat conditions, for example, neuromuscular disorders characterized by hyperactive skeletal muscles. Conditions that have been treated with botulinum toxin include hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, cerebral

palsy, cervical dystonia, migraine headaches, strabismus, temperomandibular joint disorder, and various types of muscle cramping and spasms. More recently the muscle-paralyzing effects of botulinum toxin have been taken advantage of in therapeutic and cosmetic facial applications such as treatment of wrinkles, frown lines, and other results of spasms or contractions of facial muscles.

[0013] In all treatments currently used, the botulinum toxin is administered by carefully controlled or monitored injection, creating large wells of toxin at the treatment site. A few scattered references to topical treatment are present in the literature. For example, assertions that botulinum toxin may be applied topically are made in U.S. patent 6,063,768 of Eric R. First, but no information is given as to how this may be accomplished. In another patent in which First is named as an inventor, U.S. patent 6,087,327 (of Pearce and First), mention is made that the botulinum toxin may be topically administered by solubilization in normal phosphate buffer containing gelatin stabilizer and administered topically into the nasal cavity of a dog. The patent cites a publication by Shaari et al., Otolaryngol. Head Neck Surg. **112**; 566)1995) in which such an experiment was conducted. German published patent application 198 52 981 describes topical compositions containing botulinum toxin and dimethyl sulfoxide for treatment of hyperhidosis. An example in which a single patient was treated is included.

[0014] US patent 5,670,484 (Binder) describes the use of botulinum toxin in treating cutaneous cell-proliferative disorders (for example, psoriasis and dermatitis) using neurotoxins, including botulinum toxin. The patent asserts that compositions may be applied topically, but no examples of suitable formulations are given, and in the test examples the botulinum toxin was administered by injection. U.S. published application 2003/0113349 (Coleman III) represents that topical formulations containing botulinum toxin may be used to treat hyperactive glandular conditions in the skin. However, the description relates to conditions in cutaneous glands and does not discuss transdermal applications. In addition, like most of the publications discussed here, it contains no working examples. Finally, U.S. published application 2004/0009180 (Donovan) discloses the use of botulinum toxin in topical treatments for a number of conditions, some of which apparently involve transdermal delivery, for instance, topical application to relax muscles. The publication states that transdermal delivery is accomplished by use of an enhancing agent. Agents said to be suitable for this purpose include various alcohols, including polyalcohols, amines, amides,

transferomes and liposomes. The examples are all written in the present tense, indicating that some or all may be conceptual rather than empirical.

[0015] For the most part, therefore, these publications disclose concepts, but provide little information on the actual production of compositions or the use of effective transdermal delivery of botulinum toxin for therapeutic or other purposes. However, topical application of botulinum toxin would provide for a safer and more desirable treatment alternative due to the painless nature of application, the larger treatment surface area that can be covered, the ability to formulate a pure toxin with higher specific activity, the reduced training necessary for applying the botulinum therapeutic, the smaller doses that would be necessary to produce the desired effect, and the lack of a requirement for large wells of toxin to reach a therapeutic clinical result. An effective means for transdermal delivery of botulinum toxin, as well as an effective means for administering botulinum toxin to treat or prevent a number of conditions that does not require injection is thus highly desirable.

BRIEF SUMMARY OF THE INVENTION

[0016] In one aspect, this invention relates to a composition comprising a botulinum toxin (as defined herein) and a carrier comprising a positively charged "backbone" having positively charged branching or "efficiency" groups, as described herein. Most preferably the positively charged carrier is a long-chain positively charged polypeptide or a positively charged nonpeptidyl polymer, for example, a polyalkyleneimine. The invention further relates to a method for producing a biologic effect such as muscle paralysis, reducing hypersecretion or sweating, treating neurologic pain or migraine headache, reducing muscle spasms, preventing or reducing acne, or reducing or enhancing an immune response, by topically applying an effective amount of such a composition, preferably to the skin, of a subject or patient in need of such treatment. The invention also relates to a method for producing an aesthetic or cosmetic effect, for example by topical application of botulinum toxin to the face instead of by injection into facial muscles.

[0017] This invention also provides kits for preparing or formulating a composition that comprises the carrier and the botulinum toxin, as well as such additional items that are needed to produce a usable formulation, or a premix that may in turn be used to produce such a formulation. Alternatively the kit comprises means for separately but in conjunction administering the botulinum toxin and the carrier to a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **Figure 1** represents the results of an experiment demonstrating efficiency of transdermal delivery of botulinum toxin using a composition of the invention comprising a peptide backbone.

[0019] **Figure 2** is a photograph depicting the state of the hind limbs of a mouse in which the area of one limb was treated with a composition of the invention and the area of the other was treated with another botulinum toxin-containing composition that did not contain a carrier according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0020] This invention provides compositions and methods for delivery, particularly transdermal delivery, of a botulinum toxin by topical application of an appropriate formulation.

[0021] According to the present invention, a positively charged carrier molecule having efficiency groups, as described herein, has been found suitable as a transport system for a botulinum toxin, enabling that toxin to be administered transdermally to muscles and/or other skin-associated structures. The transport occurs without covalent modification of the botulinum toxin.

[0022] By "positively charged" is meant that the carrier has a positive charge under at least some solution-phase conditions, more preferably under at least some physiologically compatible conditions More specifically, "positively charged" as used herein, means that the group in question contains functionalities that are charged under all pH conditions, for instance, a quaternary amine, or contains a functionality which can acquire positive charge under certain solution-phase conditions, such as pH changes in the case of primary amines. More preferably, "positively charged" as used herein refers to those groups that have the behavior of associating with anions over physiologically compatible conditions. Polymers with a multiplicity of positively-charged moieties need not be homopolymers, as will be apparent to one skilled in the art. Other examples of positively charged moieties are well known in the prior art and can be employed readily, as will be apparent to those skilled in the art.

[0023] In one embodiment, the carrier comprises a positively charged backbone that is a polypeptide (e.g., polylysine, polyarginine, polyornithine, polyhomoarginine, and the like)

having multiple positively charged side-chain groups. Preferably, the polypeptide has a molecular weight of from about 10,000 to about 1,500,000, more preferably from about 25,000 to about 1,200,000, most preferably from about 100,000 to about 1,000,000. In another embodiment the carrier comprises a positively charged backbone that is a nonpeptidyl polymer that may be a heteropolymer or a homopolymer. Preferably, this type of backbone is a polyalkyleneimine having a molecular weight of from about 10,000 to about 2,500,000, preferably from about 100,000 to about 1,800,000, and most preferably from about 500,000 to about 1,400,000. Such polyalkyleneimines include polyethylene- and polypropyleneimines.

[0024] In either case, the positively charged carrier includes attached positively charged branching or efficiency groups, comprising $-(gly)_{n1}-(arg)_{n2}$, HIV-TAT or fragments thereof, or Antennapedia PDT (protein transduction domain) groups, in which the subscript n1 is an integer of from 0 to 20, more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is independently an odd integer of from about 5 to about 25, more preferably from about 7 to about 17, and most preferably from about 7 to about 13. Other preferred embodiments are those in which the branching or efficiency groups are HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$, or $(gly)_p$-RKKRRQRRR-$(gly)_q$, wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the carrier molecule via either the C-terminus or the N-terminus of the fragment. The side branching groups can have either the D- or L-form (R or S configuration) at the center of attachment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5. In another preferred embodiment the positively charged side chain or branching group is the Antennapedia (Antp) protein transduction domain (PTD), or a fragment of such group that retains activity. These are known in the art, for instance, from Console et al., J. Biol. Chem. 278:35109 (2003). Preferably the positively charged carrier includes side-chain positively charged branching groups in an amount of at least about 0.05 %, as a percentage of the total carrier weight, preferably from about 0.05 to about 45 weight %, and most preferably from about 0.1 to about 30 weight %. For positively charged branching groups having the formula $-(gly)_{n1}-(arg)_{n2}$, the most preferred amount is from about 0.1 to about 25 %.

[0025] In a particularly preferred embodiment, the positively charged backbone is a polylysine with positively charged branching groups attached to the lysine side-chain amino

groups. The polylysine used in this particularly preferred embodiment can be any of the commercially available (Sigma Chemical Company, St. Louis, Missouri, USA, for example) polylysines such as, for example, polylysine having MW > 70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW 150,000 to 300,000 and polylysine having MW > 300,000. However, preferably the polylysine has MW of at least about 10,000, up to about 1,500,000, preferably from about 25,000 to about 1,200,000, and most preferably from about 100,000 to about 1,000,000. Preferred positively charged branching groups or efficiency groups include, for example, –gly-gly-gly-arg-arg-arg-arg-arg-arg-arg (-Gly_3Arg_7), Antennapedia PTD groups, or HIV-TAT, or fragments of either.

[0026] In another preferred embodiment the positively charged backbone comprises a long chain polyalkyleneimine such as a polyethyleneimine or polypropyleneimine. Such a backbone would have a molecular weight of from about 10,000 to about 1,500,000, preferably from about 25,000 to about 1,200,000, and most preferably from about 100,000 to about 1,000,000.

[0027] The term "botulinum toxin" as used herein is meant to refer to any of the known types of botulinum toxin, whether produced by the bacterium or by recombinant techniques, as well as any such types that may be subsequently discovered including engineered variants or fusion proteins. The term "botulinum toxin" also encompasses fragments thereof retaining light chain activity. As mentioned above, at the present time, seven immunologically distinct botulinum neurotoxins have been characterized, namely botulinum neurotoxin serotypes A, B, C, D, E, F and G, each of which is distinguished by neutralization with type-specific antibodies. The botulinum toxin serotypes are available from Sigma-Aldrich and from Metabiologics, Inc. (Madison, Wisconsin), as well as from other sources. The different serotypes of botulinum toxin vary in the animal species that they affect and in the severity and duration of the paralysis they evoke. At least two types of botulinum toxin, types A and B, are available commercially in formulations for treatment of certain conditions. Type A, for example, is contained in preparations of Allergan having the trademark BOTOX® and of Ipsen having the trademark DYSPORT®, and type B is contained in preparations of Elan having the trademark MYOBLOC®.

[0028] The botulinum toxin used in the compositions of this invention can alternatively be a botulinum toxin derivative, that is, a compound that has botulinum toxin activity but contains one or more chemical or functional alterations on any part or on any chain relative to

naturally occurring or recombinant native botulinum toxins. For instance, the botulinum toxin may be a modified neurotoxin, that is a neurotoxin which has at least one of its amino acids deleted, modified or replaced, as compared to a native, or the modified neurotoxin can be a recombinant produced neurotoxin or a derivative or fragment thereof. For instance, the botulinum toxin may be one that has been modified in a way that, for instance, enhances its properties or decreases undesirable side effects, but that still retains the desired botulinum toxin activity. The botulinum toxin may be any of the botulinum toxin complexes produced by the bacterium, as described above. Alternatively the botulinum toxin may be a toxin prepared using recombinant or synthetic chemical techniques, e.g. a recombinant peptide, a fusion protein, or a hybrid neurotoxin, for example prepared from subunits or domains of different botulinum toxin serotypes (see U.S. patent 6,444,209, for instance). The botulinum toxin may also be a portion of the overall molecule that has been shown to possess the necessary botulinum toxin activity, and in such case may be used per se or as part of a combination or conjugate molecule, for instance a fusion protein. Alternatively, the botulinum toxin may be in the form of a botulinum toxin precursor, which may itself be non-toxic, for instance a nontoxic zinc protease that becomes toxic on proteolytic cleavage. In preferred embodiments, the botulinum toxin is not covalently attached to a polyanion.

[0029] This invention also contemplates the general use of combinations and mixtures of botulinum toxins, though due to their differing nature and properties, mixtures of botulinum toxin serotypes are not generally administered at this time.

[0030] Compositions of this invention are preferably in the form of products to be applied to the skin or epithelium of subjects or patients, i.e. humans or other mammals in need of the particular treatment. The term "in need" is meant to include both pharmaceutical or health-related needs, for example, treating conditions involving undesirable facial muscle spasms, as well as cosmetic and subjective needs, for example, altering or improving the appearance of facial tissue. In general the compositions are prepared by mixing the botulinum toxin with the carrier, and usually with one or more additional pharmaceutically acceptable carriers or excipients. In their simplest form they may contain a simple aqueous pharmaceutically acceptable carrier or diluent, such as buffered saline. However, the compositions may contain other ingredients typical in topical pharmaceutical or cosmeceutical compositions, that is, a dermatologically or pharmaceutically acceptable carrier, vehicle or medium, i.e. a carrier, vehicle or medium that is compatible with the tissues to which they will be applied. The term "dermatologically or pharmaceutically acceptable," as used herein, means that the

compositions or components thereof so described are suitable for use in contact with these tissues or for use in patients in general without undue toxicity, incompatibility, instability, allergic response, and the like. As appropriate, compositions of the invention may comprise any ingredient conventionally used in the fields under consideration, and particularly in cosmetics and dermatology. The compositions also may include a quantity of a small anion, preferably a polyvalent anion, for example, phosphate, aspartate, or citrate.

[0031] In terms of their form, compositions of this invention may include solutions, emulsions (including microemulsions), suspensions, creams, lotions, gels, powders, or other typical solid or liquid compositions used for application to skin and other tissues where the compositions may be used. Such compositions may contain, in addition to the botulinum toxin and carrier, other ingredients typically used in such products, such as antimicrobials, moisturizers and hydration agents, penetration agents, preservatives, emulsifiers, natural or synthetic oils, solvents, surfactants, detergents, gelling agents, emollients, antioxidants, fragrances, fillers, thickeners, waxes, odor absorbers, dyestuffs, coloring agents, powders, viscosity-controlling agents and water, and optionally including anesthetics, anti-itch actives, botanical extracts, conditioning agents, darkening or lightening agents, glitter, humectants, mica, minerals, polyphenols, silicones or derivatives thereof, sunblocks, vitamins, and phytomedicinals.

[0032] Compositions according to this invention may be in the form of controlled-release or sustained-release compositions, wherein the botulinum toxin and the carrier are encapsulated or otherwise contained within a material such that they are released onto the skin in a controlled manner over time. The botulinum toxin and carrier may be contained within matrixes, liposomes, vesicles, microcapsules, microspheres and the like, or within a solid particulate material, all of which is selected and/or constructed to provide release of the botulinum toxin [them] over time. The botulinum toxin and the carrier may be encapsulated together (e.g., in the same capsule) or separately (in separate capsules).

[0033] Botulinum toxin can be delivered to muscles underlying the skin, or to glandular structures within the skin, in an effective amount to produce paralysis, produce relaxation, alleviate contractions, prevent or alleviate spasms, reduce glandular output, or other desired effects. Local delivery of the botulinum toxin in this manner could afford dosage reductions, reduce toxicity and allow more precise dosage optimization for desired effects relative to injectable or implantable materials.

[0034] The compositions of the invention are applied so as to administer an effective amount of the botulinum toxin. The term "effective amount" as used herein means an amount of a botulinum toxin as defined above that is sufficient to produce the desired muscular paralysis or other biological or aesthetic effect, but that implicitly is a safe amount, i.e. one that is low enough to avoid serious side effects. Desired effects include the relaxation of certain muscles with the aim of, for instance, decreasing the appearance of fine lines and/or wrinkles, especially in the face, or adjusting facial appearance in other ways such as widening the eyes, lifting the corners of the mouth, or smoothing lines that fan out from the upper lip, or the general relief of muscular tension. The last-mentioned effect, general relief of muscular tension, can be effected in the face or elsewhere. The compositions of the invention may contain an appropriate effective amount of the botulinum toxin for application as a single-dose treatment, or may be more concentrated, either for dilution at the place of administration or for use in multiple applications. Through the use of the positively charged carriers of this invention, a botulinum toxin can be administered transdermally to a subject for treating conditions such as undesirable facial muscle or other muscular spasms, hyperhidrosis, acne, or conditions elsewhere in the body in which relief of muscular ache or spasms is desired. The botulinum toxin is administered topically for transdermal delivery to muscles or to other skin-associated structures. The administration may be made, for example, to the legs, shoulders, back (including lower back), axilla, palms, feet, neck, groin, dorsa of the hands or feet, elbows, upper arms, knees, upper legs, buttocks, torso, pelvis, or any other part of the body where administration of the botulinum toxin is desired.

[0035] Administration of botulinum toxin may also be carried out to treat other conditions, including treating of neurologic pain, prevention or reduction of migraine headache or other headache pain, prevention or reduction of acne, prevention or reduction of dystonia or dystonic contractions (whether subjective or clinical), prevention or reduction of symptoms associated with subjective or clinical hyperhidrosis, reducing hypersecretion or sweating, reducing or enhancing immune response, or treatment of other conditions for which administration of botulinum toxin by injection has been suggested or performed.

[0036] Administration of botulinum toxin or other therapeutic proteins described herein may also be carried out for immunization-related purposes. For instance, the complexed botulinum toxin or other protein allows an altered route of administration and may be useful to reduce immune response to antigens to that protein, and thus facilitate repeat administration without immune-related reduction in activity. Alternately, the complex can be

prepared and applied topically to enhance an immune response, for example to provide immunizations respecting various proteins, for example, for childhood immunizations without injections.

[0037] Surprisingly, administration of botulinum toxin described herein may also be carried out to reduce immune responses. The present invention allows a botulinum toxin to be delivered by an altered route of administration and changes the complex antigen presentation of the agent, and may thus be useful to reduce immune response to antigens to botulinum toxin, and thus facilitate repeat administration without immune-related reduction in activity. For use in connection with immune-related activity, an "effective amount" refers to an amount of the botulinum toxin sufficient to allow a subject to mount an immune response to the botulinum toxin after application or a series of applications of it.

[0038] Most preferably, the compositions are administered by or under the direction of a physician or other health care professional. They may be administered in a single treatment or in a series of periodic treatments over time. For transdermal delivery of botulinum toxin for the purposes mentioned above, a composition as described above is applied topically to the skin at a location or locations where the effect is desired. Because of its nature, most preferably the amount of botulinum toxin applied should be applied with care, at an application rate and frequency of application that will produce the desired result without producing any adverse or undesired results. Accordingly, for instance, topical compositions of the invention should be applied at a rate of from about 1U to about 20,000U, preferably from about 1U to about 10,000U botulinum toxin per cm^2 of skin surface. Higher dosages within these ranges could preferably be employed in conjunction with controlled release materials, for instance, or allowed a shorter dwell time on the skin prior to removal.

[0039] This invention also comprises devices for transdermal transmission of a botulinum toxin that contain a composition that in turn comprises a carrier that has a positively charged backbone with attached branching groups as defined herein, and a botulinum toxin. Such devices may be as simple in construction as a skin patch, or may be a more complicated device that includes means for dispensing and monitoring the dispensing of the composition, and optionally means for monitoring the condition of the subject in one or more aspects, including monitoring the reaction of the subject to the substances being dispensed.

[0040] The compositions, both in general, and in such devices, can be pre-formulated or pre-installed in the device as such, or can be prepared later, for example using a kit that

contains the two ingredients (botulinum toxin and carrier) for combining at or prior to the time of application. The amount of carrier molecule or the ratio of it to the botulinum toxin will depend on which carrier is chosen for use in the composition in question. The appropriate amount or ratio of carrier molecule in a given case can readily be determined, for example, by conducting one or more experiments such as those described below.

[0041] In general, the invention also comprises a method for administering a botulinum toxin to a subject or patient in need thereof, comprising topically administering an effective amount of the botulinum toxin in conjunction with a carrier comprising a positively charged backbone with attached positively charged branching groups, as described herein. By "in conjunction with" is meant that the two components (botulinum toxin and carrier) are administered in a combination procedure, which may involve either combining them in a composition, which is then administered to the subject, or administering them separately, but in a manner such that they act together to provide the requisite delivery of an effective amount of the therapeutic protein. For example, a composition containing the carrier may first be applied to the skin of the subject, followed by applying a skin patch or other device containing the botulinum toxin. The botulinum toxin may be incorporated in dry form in a skin patch or other dispensing device and the positively charged carrier may be applied to the skin surface before application of the patch so that the two act together, resulting in the desired transdermal delivery. In that sense, thus, the two substances (carrier and botulinum toxin) act in combination or perhaps interact to form a composition or combination in situ. Accordingly, the invention also comprises a kit that includes both a device for dispensing botulinum toxin via the skin and a liquid, gel, cream or the like that contains the carrier or backbone, and that is suitable for applying to the skin or epithelium of a subject. Kits for administering the compositions of the inventions, either under direction of a health care professional or by the patient or subject, may also include a custom applicator suitable for that purpose.

[0042] The compositions, kits and methods of this invention allow for the delivery of a more pure botulinum toxin with higher specific activity and potentially improved pharmacokinetics. In addition, the carrier can act as a stabilizer, reducing the need for foreign accessory proteins (e.g., human serum albumin ranging from 400-600 mg or recombinant serum albumin ranging from 250-500 mg) and/or polysaccharide stabilizers, and can afford beneficial reductions in immune responses to the botulinum toxin. In addition, the compositions are suitable for use in physiologic environments with pH ranging from about

4.5 to about 6.3, and may thus have such a pH. The compositions according to this invention may be stored either at room temperature or under refrigerated conditions.

[0043] The following are representative examples of the invention. They demonstrate delivery of functional botulinum neurotoxin complexes across skin without requiring covalent modification of the neurotoxin to be delivered.

Example 1. Transport of a botulinum toxin in vivo using a peptidyl carrier.

[0044] This experiment demonstrates the use of a peptidyl carrier to transport a large complex containing an intact labeled protein botulinum toxin across intact skin after a single time administration relative to controls.

Backbone selection:

[0045] The positively charged backbone was assembled by conjugating $-Gly_3Arg_7$ to polylysine MW 112,000 via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is conjugated to a $-Gly_3Arg_7$). The modified backbone was designated "KNR". The control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, MO) of the same size and from the same lot.

Therapeutic agent:

[0046] Botox® brand of botulinum toxin A (Allergan) was selected for this experiment. It has a molecular weight of approximately 150,000.

Preparation of samples

[0047] The botulinum toxin was reconstituted according to the manufacturer's instructions. An aliquot of the protein was biotinylated with a calculated 12-fold molar excess of sulfo-NHS-LC biotin (Pierce Chemical). The labeled product was designated "Btox-b".

[0048] In each case, an excess of polycation was employed to assemble a final complex that has an excess of positive charge as in delivery of highly negative large nucleic acid complexes. A net neutral or positive charge prevents repulsion of the protein complex from highly negative cell surface proteoglycans and extracellular matrix. Btox-b dose was

standardized across all groups, as was total volume and final pH of the composition to be applied topically. Samples were prepared as follows:

[0049] Group labeled "JMW-7": 2.0 units of Btox-b per aliquot (i.e. 20 U total) and peptidyl carrier KNR at a calculated MW ratio of 4:1 were mixed to homogeneity and diluted to 200 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 1.8 ml of Cetaphil® lotion and aliquoted in 200 microliter portions.

[0050] Group labeled "JMW-8": 2.0 units of Btox-b per aliquot (i.e. 20 U total) and K at a charge ratio of 4:1 were mixed to homogeneity and diluted to 200 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 1.8 ml of Cetaphil and aliquoted in 200 microliter portions.

Animal experiments to determine transdermal delivery efficiencies after single time treatment with peptidyl carriers and labeled Btox:

[0051] Animals were anesthetized via inhalation of isoflurane during application of treatments. After being anesthetized, C57 black 6 mice (n=4 per group) underwent topical application of metered 200 microliter dose of the appropriate treatment applied to the cranial portion of dorsal back skin (selected because the mouse cannot reach this region with mouth or limbs). Animals did not undergo depilation. At 30 minutes after the initial treatment, mice were euthanized via inhalation of CO_2, and treated skin segments were harvested at full thickness by blinded observers. Treated segments were divided into three equal portions; the cranial portion was fixed in 10% neutral buffered formalin for 12-16 hours then stored in 70% ethanol until paraffin embedding. The central portion was snap-frozen and employed directly for biotin visualization by blinded observers as summarized below. The treated caudal segment was snap frozen for solubilization studies.

[0052] Biotin visualization was conducted as follows. Briefly, each section was immersed for 1 hour in NeutrAvidin® buffer solution. To visualize alkaline phosphatase activity, cross sections were washed in saline four times then immersed in NBT/BCIP (Pierce Scientific) for 1 hour. Sections were then rinsed in saline and photographed in entirety on a Nikon E600 microscope with plan-apochromat lenses.

Data handling and statistical analysis:

[0053] Total positive staining was determined by blinded observer via batch image analysis using Image Pro Plus software (Media Cybernetics, Silver Spring, MD) and was normalized to total cross-sectional area to determine percent positive staining for each. Mean and

standard error were subsequently determined for each group with analysis of significance at 95% confidence in one way ANOVA repeated measures using Statview software (Abacus, Berkeley, CA).

Results:

[0054] The mean cross-sectional area positive for biotinylated botulinum toxin was reported as percent of total area after single-time topical administration of Btox-b with either KNR ("EB-Btox") or K ("nl"). The results are presented in the following Table 1 and are illustrated in Figure 1. In Figure 1, the area positive for label was determined as percent of total area after three days of once daily treatment with "EB-Btox" which contained Btox-b and the peptidyl carrier KNR and "nl", which contained Btox-b with polycation K as a control. Mean and standard error are depicted for each group.

Table 1. Mean and standard error for labeled botulinum toxin area as percent of total cross-section after single time topical administration of Btox-b with KNR (JMW-7) or K (JMW-8) for 30 minutes.

Group	Mean	Std. Error
JMW-7	33	5.333334
JMW-8	8.666667	0.333334

P=0.0001 (Significant at 99%)

Example 2. Therapeutic efficacy of a topical botulinum toxin preparation with a peptidyl carrier.

[0055] Example 1 demonstrated that the peptidyl transdermal carrier allowed efficient transfer of botulinum toxin after topical administration in a murine model of intact skin. However, this experiment did not indicate whether the complex protein botulinum toxin was released in a functional form after translocation across skin. The following experiment was thus constructed to evaluate whether botulinum toxin can be therapeutically delivered across intact skin as a topical agent using this peptidyl carrier (again, without covalent modification of the protein).

[0056] The positively charged backbone was again assembled by conjugating $-Gly_3Arg_7$ to polylysine MW 112,000 via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is

conjugated to a $-Gly_3Arg_7$). The modified backbone was designated "KNR". Control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, MO) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in Example 1, and was prepared in the same manner. Samples were prepared as follows:

[0057] Group labeled "JMW-9": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and peptidyl carrier KNR at a calculated MW ratio of 4:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

[0058] Group labeled "JMW-10": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and K at a charge ratio of 4:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

[0059] Group labeled "JMW-11": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) without polycation was diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

Animal experiments to determine therapeutic efficacy after single time treatment with peptidyl carriers and botulinum toxin:

[0060] Animals were anesthetized via inhalation of isoflurane during application of treatments. After being anesthetized, C57 black 6 mice (n=4 per group) underwent topical application of metered 400 microliter dose of the appropriate treatment applied uniformly from the toes to the mid-thigh. Both limbs were treated, and treatments were randomized to either side. Animals did not undergo depilation. At 30 minutes after the initial treatment, mice were evaluated for digital abduction capability according to published digital abduction scores for foot mobility after botulinum toxin administration [Aoki, KR. *A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice*. Toxicon. 2001 Dec; 39(12): 1815-20]. Mouse mobility was also subjectively assessed.

Data handling and statistical analysis:

[0061] Digital abduction scores were tabulated independently by two blinded observers. Mean and standard error were subsequently determined for each group with analysis of

significance at 95% confidence in one way ANOVA repeated measures using Statview software (Abacus, Berkeley, CA).

Results:

[0062] Mean digital abduction scores after single-time topical administration of botulinum toxin with KNR ("JMW-9"), K ("JMW-10") or diluent without polycation ("JMW-11"), are presented in table 2 and illustrated in the representative photomicrograph of figure 2 below. The peptidyl carrier KNR afforded statistically significant functional delivery of the botulinum toxin across skin relative to both controls, which were comparable to one another. Additional independent repetitions (total of three independent experiments all with identical conclusions in statistically significant paralysis from topical botulinum toxin with KNR but not controls) of the present experiment confirmed the present findings and revealed no significant differences between topical botulinum toxin with or without K (i.e. both controls). Interestingly, the mice consistently ambulated toward a paralyzed limb (which occurred in 100% of treated animals and 0% of controls from either control group). As shown in Figure 2, a limb treated with botulinum toxin plus the control polycation polylysine or with botulinum toxin without polycation ("Botox alone") can mobilize digits (as a defense mechanism when picked up), but the limbs treated with botulinum toxin plus the peptidyl carrier KNR ("Essentia Botox lotion") could not be moved.

Table 2 . Digital abduction scores 30 minutes after single-time topical application of botulinum toxin with the peptidyl carrier KNR ("JMW-9"), with a control polycation K ("JMW-10"), or alone ("JMW-11").

Group	Mean	Std. Error
JMW-9	3.333	0.333
JMW-10	0.333	0.333
JMW-11	0.793	0.300

P=0.0351 (Significant at 95%)

Conclusions:

[0063] This experiment serves to demonstrate that the peptidyl transdermal carrier can transport a therapeutically effective amount of botulinum therapeutic across skin without covalent modification of the therapeutic. The experiment also confirms that botulinum toxin does not function when applied topically in controls.

Example 3. Therapeutic efficacy of a topical botulinum toxin preparation with a nonpeptidyl Carrier.

[0064] This experiment demonstrates the performance of a non-peptidyl carrier in the invention.

Methods:

Backbone selection:

[0065] The positively charged backbone was assembled by conjugating $-Gly_3Arg_7$ to polyethyleneimine (PEI) MW 1,000,000 via the carboxyl of the terminal glycine to free amines of the PEI side chains at a degree of saturation of 30% (i.e., 30 out of each 100 lysine residues is conjugated to a $-Gly_3Arg_7$). The modified backbone was designated "PEIR" to denote the large nonpeptidyl carrier. Control polycation was unmodified PEI (designated "PEI", Sigma Chemical Co., St. Louis, MO) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in example 1.

[0066] Botulinum toxin was reconstituted from the Botox product according to the manufacturer's instructions. In each case, an excess of polycation was employed to assemble a final complex that had an excess of positive charge as in delivery of highly negative large nucleic acid complexes. A net neutral or positive charge prevents repulsion of the protein complex from highly negative cell surface proteoglycans and extracellular matrix. The botulinum toxin dose was standardized across all groups as was total volume and final pH of the composition to be applied topically. Samples were prepared as follows:

[0067] Group labeled "AZ": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and the nonpeptidyl carrier PEIR in ultrapure form at a calculated MW ratio of 5:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

[0068] Group labeled "BA": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and PEI at a charge ratio of 5:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

Animal experiments to determine therapeutic efficacy after single time treatment:

[0069] Animals were anesthetized via inhalation of isoflurane during application of treatments. After being anesthetized, C57 black 6 mice (n=3 per group) underwent topical application of metered 400 microliter dose of the appropriate treatment applied uniformly from the toes to the mid-thigh. Both limbs were treated, and treatments were randomized to either side. Animals did not undergo depilation. At 30 minutes after the initial treatment, mice were evaluated for digital abduction capability according to published digital abduction scores for foot mobility after botulinum toxin administration [Aoki, KR. *A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice*. Toxicon. 2001 Dec; 39(12): 1815-20]. Mouse mobility was also subjectively assessed.

Data handling and statistical analysis:

[0070] Digital abduction scores were tabulated independently by two blinded observers. Mean and standard error were subsequently determined for each group with analysis of significance at 95% confidence in one way ANOVA repeated measures using Statview software (Abacus, Berkeley, CA).

Results:

[0071] Mean digital abduction scores after single-time topical administration of botulinum toxin with ultrapure PEIR ("AZ"), or control polycation PEI ("BA"), are presented in table 3 and repetition presented as table 4 (single independent repetition for this experiment). The nonpeptidyl carrier PEIR afforded statistically significant functional delivery of botulinum toxin across skin relative to controls. As before, animals were observed to walk in circles toward the paralyzed limbs.

Table 3. Digital abduction scores 30 minutes after single-time topical administration of Botox with ultrapure PEIR ("AZ"), or control polycation PEI ("BA"). Mean and standard error are presented.

Group	Mean	Std. Error
BA	0.833	0.307
AZ	3.917	0.083

P=0.0002 (Significant at 99%)

Table 4. Digital abduction scores 30 minutes after single-time topical administration of Botox with ultrapure PEIR ("AZ1"), or control polycation PEI ("BA1"). Mean and standard error are presented.

Group	Mean	Std. Error
BA1	0.333	0.211
AZ1	3.833	0.167

P=0.0001 (Significant at 99%)

Conclusions:

[0072] This experiment demonstrated that the nonpeptidyl transdermal carrier can transport therapeutic doses of botulinum toxin across skin without prior covalent modification of the botulinum toxin. These findings complement those with peptidyl transfer agents. The option of using a nonpeptidyl or a peptidyl carrier to achieve the therapeutic effect will allow tailoring to specific circumstances, environments, and methods of application and add to the breadth of the transdermal delivery platform of this invention.

[0073] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A composition comprising a botulinum toxin and a carrier comprising a polymeric backbone having attached positively charged branching groups, wherein the association between the carrier and the botulinum toxin is non-covalent.

2. A composition according to claim 1 in which the botulinum toxin is a botulinum toxin derivative.

3 A composition according to claim 1 in which the botulinum toxin comprises a recombinant botulinum toxin.

4. A composition according to claim 1 in which the botulinum toxin comprises a modified botulinum toxin.

5. A composition according to claim 1 in which the botulinum toxin is selected from botulinum toxin serotypes A, B, C, D, E, F and G.

6. A composition according to claim 5 in which the botulinum toxin is botulinum toxin A.

7 A composition according to claim 5 in which the botulinum toxin is botulinum toxin B.

8. A composition according to claim 5 in which the botulinum toxin is botulinum toxin C_1.

9. A composition according to claim 5 in which the botulinum toxin is botulinum toxin D.

10. A composition according to claim 5 in which the botulinum toxin is botulinum toxin E.

11. A composition according to claim 1 in which the carrier comprises a polypeptide having attached positively charged branching groups independently selected from $-(gly)_{n1}-(arg)_{n2}$, HIV-TAT, Antennapedia PTD, and fragments of HIV-TAT or of Antennapedia PTD or mixtures thereof, in which the subscript n1 is an integer of from 0 to about 20, and the subscript n2 is independently an odd integer of from about 5 to about 25.

12. A composition according to claim 11 in which the carrier comprises a polypeptide having positively charged branching groups independently selected from $-(gly)_{n1}-(arg)_{n2}$ in which the subscript n1 is an integer of from about 0 to about 20 and the subscript n2 is independently an odd integer of from about 5 to about 25.

13. A composition according to claim 12 in which the subscript n1 is an integer of from 0 to about 8.

14. A composition according to claim 12 in which the subscript n1 is an integer of from about 2 to about 5

15. A composition according to claim 12 in which the subscript n2 is an odd integer of from about 7 to about 17.

16. A composition according to claim 12 in which the subscript n2 is an odd integer from about 7 to about 13.

17. A composition according to claim 11 in which the carrier comprises a polypeptide having attached positively charged branching groups selected from HIV-TAT and fragments thereof.

18. A composition according to claim 17 in which the branching groups are positively charged HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$, or $(gly)_p$-RKKRRQRRR-$(gly)_q$, wherein the subscripts p and q are each independently an integer of from 0 to 20.

19. A composition according to claim 1 in which the positively charged branching groups comprise at least about 0.05 % by weight of the total carrier weight.

20. A composition according to claim 1 in which the positively charged branching groups comprise from about 0.5% to about 45% by weight of the total carrier weight.

21. A composition according to claim 1 in which the positively charged branching groups comprise from about 0.1 % to about 30% by weight of the total carrier weight.

22. A composition according to claim 1 in which the backbone comprises a positively charged polypeptide.

23. A composition according to claim 22 in which the backbone comprises a positively charged polylysine.

24. A composition according to claim 23 in which the polylysine has a molecular weight of from about 10,000 to 1.500,000.

25. A composition according to claim 23 in which the polylysine has a molecular weight of from about 25,000 to about 1,200,000.

26. A composition according to claim 23 in which the polylysine has a molecular weight of from about 100,000 to about 1,000,000.

27. A composition according to claim 1 in which the backbone comprises a positively charged nonpeptidyl carrier.

28. A composition according to claim 27 in which the backbone comprises a positively charged polyalkyleneimine.

29. A composition according to claim 28 in which the polyalkyleneimine is a polyethyleneimine.

30. A composition according to claim 29 in which the polyethyleneimine has a molecular weight of from about 10,000 to about 2,500,000.

31. A composition according to claim 29 in which the polyethyleneimine has a molecular weight of from about 100,000 to about 1,800,000,

32. A composition according to claim 29 in which the polyethyleneimine has a molecular weight of from about 500,000 to about 1,400,000.

33. A composition according to claim 1 having a pH of from about 4.5 to about 6.3.

34. A composition according to claim 1 that is stable when stored at room temperature or under refrigerated conditions.

35 . A controlled release composition according to claim 1.

36 . A liquid composition according to claim 1.

37. A gel composition according to claim 1.

38. A composition according to claim 1 that is a cream, lotion or ointment.

39. A composition according to claim 1 further comprising saline.

40. A composition according to claim 1 further comprising saline and a pH buffer system.

41. A kit for administration of a botulinum toxin to a subject comprising a botulinum toxin and an effective amount for transdermal delivery thereof, of a carrier comprising a polymeric backbone having attached positively charged branching groups, wherein the association between the carrier and the botulinum toxin is non-covalent.

42. A kit according to claim 41 further comprising a custom applicator.

43. A kit according to claim 42 in which the custom applicator is designed for use by a health care professional.

44. A kit according to claim 42 in which the custom applicator is designed for self-administration by a subject.

45. A kit according to claim 41 comprising a pre-formulated composition comprising the botulinum toxin and the carrier.

46. A kit according to claim 41 in which the botulinum toxin and the carrier are separately formulated for combining prior to administration.

47. A kit according to claim 41 in which the botulinum toxin is contained in a device for administering the botulinum toxin to a subject via the skin.

48. A kit according to claim 47 in which the device is a skin patch.

49. A kit for administration of a botulinum toxin to a subject comprising a device for delivering the botulinum toxin to the skin and a composition comprising a carrier comprising a polymeric backbone having attached positively charged branching groups independently selected from – $(gly)_{n1}$-$(arg)_{n2}$, HIV-TAT and fragments thereof, and Antennapedia PTD or mixtures thereof, in which the subscript n1 is an integer of from 0 to about 20, and the subscript n2 is independently an odd integer of from about 5 to about 25.

50 . A kit according to claim 49 in which the device is a skin patch.

51. A method of administering a botulinum toxin to a subject comprising topically applying to the skin or epithelium of the subject the botulinum toxin in conjunction with an effective amount of a carrier comprising a polymeric backbone having attached positively charged branching groups, wherein the association between the carrier and the botulinum toxin is non-covalent.

52. A method according to claim 51 comprising topically applying to the skin or epithelium of the subject an effective amount of a composition according to claim 1.

53. A method according to claim 51 in comprising separately applying the botulinum toxin and the carrier to the skin or epithelium of the subject.

54. A method according to claim 51 in which the botulinum toxin is administered to achieve a desired biologic effect.

55. A method according to claim 54 in which the botulinum toxin is administered to achieve an aesthetic and/or cosmetic benefit.

56. A method according to claim 54 in which the botulinum toxin is applied to reduce or prevent an immune response.

57 A method according to claim 56 in which the reduced or prevented immune response improves therapeutic response on later repeat re-administrations of the composition.

58. A method according to claim 54 in which the botulinum toxin is administered for prevention or reduction of symptoms associated with subjective or clinical hyperhidrosis.

59. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of subjective or clinical dystonic contractions or dystonia.

60 . A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with muscle spasm.

61. A method according to claim 60 in which the botulinum toxin is applied topically to the lower back of the subject, or to a portion thereof.

62. A method according to claim 60 in which the botulinum toxin is topically applied to the neck of the subject, or to a portion thereof.

63. A method according to claim 60 in which the botulinum toxin is topically applied to at least one leg of the subject, or to a portion thereof.

64. A method according to claim 51 in which the botulinum toxin is applied topically to the face of the subject, or to a portion thereof.

65. A method according to claim 51 in which the botulinum toxin is applied topically to the axilla of the subject, or to a portion thereof.

66. A method according to claim 51 in which the botulinum toxin is applied topically to the palms of the hands or to the feet of the subject, or to a portion thereof.

67. A method according to claim 51 in which the botulinum toxin is applied topically to the scalp of the subject, or to a portion thereof.

68. A method according to claim 51 in which the botulinum toxin is applied topically to the groin of the subject, or to a portion thereof.

69. A method according to claim 51 in which the composition is applied topically to the hands or feet of the subject, or to a portion thereof.

70. A method according to claim 51 in which the botulinum toxin is applied topically to the elbows, upper arms, knees, or upper legs of the subject, or to a portion thereof.

71. A method according to claim 51 in which the botulinum toxin is applied topically to the buttocks of the subject or to a portion thereof.

72. A method according to claim 51 in which the botulinum toxin is applied topically to the torso of the subject or to a portion thereof.

73. A method according to claim 51 in which the botulinum toxin is applied topically to the pelvis of the subject or to a portion thereof.

74. A method according to claim 51 in which the botulinum toxin is applied to generate or enhance an immune response.

75. A method according to claim 51 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with migraine headache.

76. A method according to claim 51 in which the botulinum toxin is applied topically for prevention or reduction of acne.

77. A method according to claim 51 in which the botulinum toxin is a botulinum toxin derivative.

78. A method according to claim 51 in which the botulinum toxin comprises a recombinant botulinum toxin.

79. A method according to claim 51 in which the botulinum toxin comprises a modified botulinum toxin.

80. A method according to claim 51 in which the botulinum toxin is selected from botulinum toxin serotypes A, B, C, D, E, F and G.

81. A method according to claim 51 in which the botulinum toxin is botulinum toxin A.

82 A method according to claim 51 in which the botulinum toxin is botulinum toxin B.

83. A method according to claim 51 in which the botulinum toxin is botulinum toxin C.

84. A method according to claim 51 in which the botulinum toxin is botulinum toxin D.

85. A method according to claim 51 in which the botulinum toxin is botulinum toxin E.

86. A method according to claim 51 in which the carrier comprises a polymeric backbone having attached positively charged branching groups independently selected from – $(gly)_{n1}$-$(arg)_{n2}$, HIV-TAT, Antennapedia PTD or mixtures thereof, and fragments of HIV-TAT or of Antennapedia PTD, in which the subscript n1 is an integer of from 0 to about 20, and the subscript n2 is independently an odd integer of from about 5 to about 25.

87. A method according to claim 86 in which the carrier comprises a polypeptide having positively charged branching groups independently selected from - $(gly)_{n1}$-$(arg)_{n2}$ in which the subscript n1 is an integer of from about 0 to about 20 and the subscript n2 is independently an odd integer of from about 5 to about 25.

88. A method according to claim 87 in which the subscript n1 is an integer of from 0 to about 8.

89. A method according to claim 87 in which the subscript n1 is an integer of from about 2 to about 5

90. A method according to claim 87 in which the subscript n2 is an odd integer of from about 7 to about 17.

91. A method according to claim 87 in which the subscript n2 is an odd integer from about 7 to about 13.

92. A method according to claim 86 in which the carrier comprises a polypeptide having attached positively charged branching groups selected from HIV-TAT and fragments thereof.

93. A method according to claim 92 in which the branching groups are positively charged HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$, or $(gly)_p$-RKKRRQRRR-$(gly)_q$, wherein the subscripts p and q are each independently an integer of from 0 to 20.

94. A method according to claim 51 in which the positively charged branching groups comprise at least about 0.05 % by weight of the total carrier weight.

95. A method according to claim 51 in which the positively charged branching groups comprise from about 0.5% to about 45% by weight of the total carrier weight.

96. A method according to claim 51 in which the positively charged branching groups comprise from about 0.1 % to about 30% by weight of the total carrier weight.

97. A method according to claim 51 in which the backbone comprises a positively charged polypeptide.

98. A method according to claim 97 in which the backbone comprises a positively charged polylysine.

99. A method according to claim 98 in which the polylysine has a molecular weight of from about 10,000 to 1.5 million.

100. A method according to claim 99 in which the polylysine has a molecular weight of from about 25,000 to about 1,200,000.

101. A method according to claim 99 in which the polylysine has a molecular weight of from about 100,000 to about 1,000,000.

102. A method according to claim 51 in which the backbone comprises a positively charged nonpeptidyl carrier.

103. A method according to claim 102 in which the positively charged nonpeptidyl polymer is polyalkyleneimine.

104. A method according to claim 103 in which the polyalkyleneimine is a polyethyleneimine.

105. A method according to claim 104 in which the polyethyleneimine has a molecular weight of from about 10,000 to about 2,500,000.

106. A method according to claim 105 in which the polyethyleneimine has a molecular weight of from about 100,000 to about 1,800,000.

107. A method according to claim 105 in which the polyethyleneimine has a molecular weight of from about 500,000 to about 1,400,000.

108. A method according to claim 51 in which the botulinum toxin comprises a recombinant botulinum toxin.

109. A method according to claim 51 in which the botulinum toxin is applied in a composition having a pH of from about 4.5 to about 6.3.

110. A method according to claim 51 in which the botulinum toxin is applied in a controlled release composition.

111 . A method according to claim 51 in which the botulinum toxin is contained in a liquid composition.

112. A method according to claim 51 in which the botulinum toxin is contained in a gel composition.

113. A method according to claim 51 in which the botulinum toxin is contained in a composition that is a cream, lotion or ointment.

114. A method according to claim 51 in which the botulinum toxin is contained in a composition further comprising saline.

115. A method according to claim 51 in which the botulinum toxin is contained in a composition further comprising saline and a pH buffer system.

116. A method according to claim 51 in which the botulinum toxin is contained in a device for dispensing the botulinum toxin, which device is applied topically to the skin or epithelium of the subject.

117. A method according to claim 116 in which the device is a skin patch.

118. A method according to claim 116 in which the device is a cell-encapsulating device.

119. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with mucous secretion.

120. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of obesity or symptoms thereof.

121. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of inflammation or symptoms thereof.

122. A method according to claim 121 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with psoriasis.

123. A method according to claim 122 in which the composition is applied in conjunction with other treatment modalities.

124. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of snoring.

125. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of cutaneous symptoms associated with diabetes.

126. A method according to claim 54 in which the botulinum toxin is applied topically for improvement of wound healing.

127. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with autonomic nerve dysfunction.

128. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with cerebral palsy.

129. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with Hashimoto's thyroiditis.

130. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with mammary gland disorders.

131. A method according to claim 54 in which the botulinum toxin is applied topically for alteration of hair growth.

132. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with parathyroid disorders.

133. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with movement disorders.

134. A method according to claim 133 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with Parkinson's disease.

135. A method according to claim 133 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with tremors.

136. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with epilepsy.

137. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with inner ear disorders.

138. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with urologic disorders.

139. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of other cholinergic-controlled secretions.

140. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with neuropshychiatric disorders.

141. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with injured muscles.

142. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with ear disorders.

143. A method according to claim 54 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with cancer.

144. A method according to claim 55 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with nerve entrapment disorders.

145. A method according to claim 55 in which the botulinum toxin is applied topically for prevention or reduction of symptoms associated with hypercalcemia.

146. A method according to claim 52 in which the botulinum toxin comprises a fusion protein.

147. A composition according to claim 5 in which the botulinum toxin is botulinum toxin F.

148. A composition according to claim 5 in which the botulinum toxin is botulinum toxin G.

149. A method according to claim 51 in which the botulinum toxin is botulinum toxin F.

150. A method according to claim 51 in which the botulinum toxin is botulinum toxin G.

COMPOSITIONS AND METHODS FOR TOPICAL APPLICATION AND TRANSDERMAL DELIVERY OF BOTULINUM TOXINS

ABSTRACT OF THE DISCLOSURE

A composition for topical application of a botulinum toxin (including botulinum toxin derivatives) comprises a botulinum toxin and a carrier comprising a polymeric backbone comprising a long-chain polypeptide or nonpeptidyl polymer having attached positively charged branching or "efficiency" groups. The invention also relates to methods for reducing muscle paralysis and other conditions that may be treated with a botulinum toxin, particularly paralysis of subcutaneous, and most particularly, facial, muscles, by topically applying an effective amount of the botulinum toxin and carrier, in conjunction, to the subject's skin or epithelium. Kits for administration are also described.

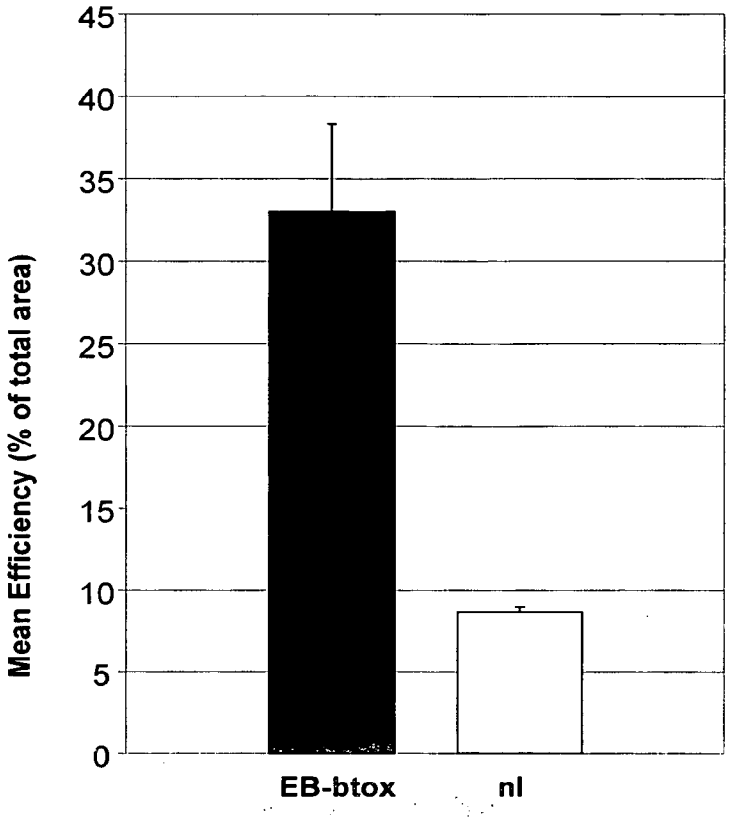

FIG. 1

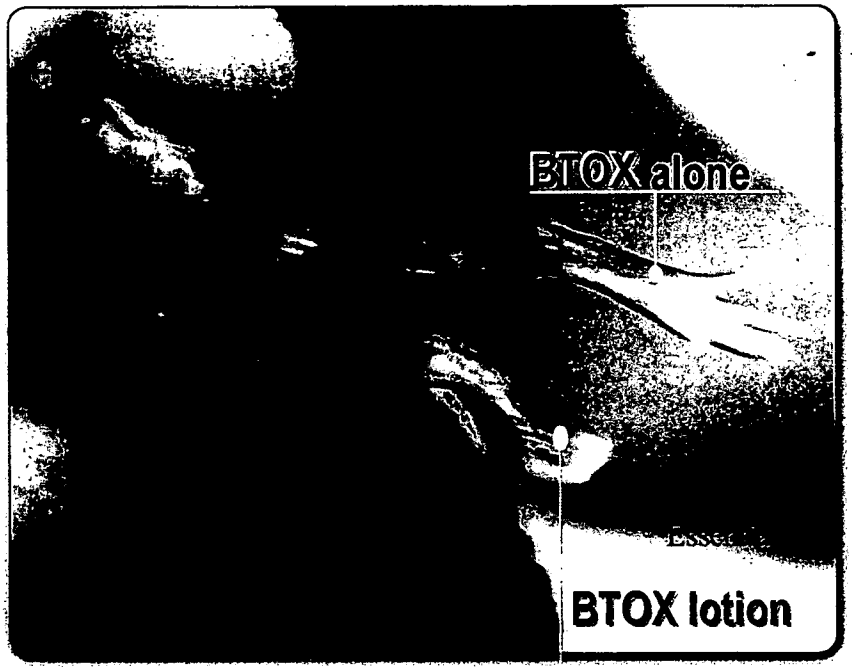

FIGURE 2